REMARKS

Currently pending in the application are claims 1-40. Claims 38-40 are amended to recite dependency to claim 7 with claim 38 reciting the group of detection of hydrolysis by any of the methods of visual observation of products of hydrolysis, fluorescence and phosphorescence, as well as spectroscopy, as discussed at pages 19-20 and Example 5 of the specification. Claims 7 and 19 are amended to recite that the hydrolysis of organophosphate is detected "such that extraction of the enzyme from the cell is not necessary to detect the hydrolysis" to determine whether the cell has incorporate the polynucleotide of interest "under conditions that do not require destruction of the cell" as demonstrated in the specification in Example 5.

Section 102 Issues

Claims 1, 2, 5, 7, 8, 11, 13, 14, 17, 19, 20, 23, 33, 34, 36 and 38 are rejected under section 102(b) as anticipated by Phillips et al. The Examiner says Phillips discloses transforming *Drosophila melanogaster* with a polynucleotide encoding organophosphate hydrolase (OPH) "and selecting transformed cells" using organophosphate paraoxon, and shows analyzing for the presence of hydrolysis by spectrophotometry. The Examiner thus concludes the references discloses all the claim limitations.

The rejection is respectfully traversed. Applicant points out that no selection of transformed cells using OPH was accomplished in the Phillips et al reference. Instead, after the cells were transformed with nucleotides encoding organophosphate hydrolase, the supernatant was assayed for OPH activity (see p. 8156, first column, first full paragraph). The transformed lines were examined for integration of *opd* sequences, presence of *opd* transcripts, OPH activity and resistance to paraoxon toxicity (p.8156,

first column, second full paragraph). The authors noted, "The level of heat shock-inducible OPH activity in these strains ranged from <1unit per mg of protein (*opd*^{328}) to >22 units per mg of protein (*opd*^{82})(Fig.4). Because of its inducibility to high levels of OPH expression, strain *opd*^{82} was used for all subsequent characterizations." (See p.8156, beginning of second column.) Thus OPH activity was assayed, and the effect of heat induction of the associated heat shock protein on OPH expression thus analyzed, but cells were not *selected* using the OPH sequences. Indeed, the control cells did not live or die depending upon the expression of OPH; rather OPH activity was simply measured and not detected. (See p.8156, column 2, second full paragraph).

The fact that OPH activity was measured, but not used to select transformed cells, is emphasized by the purpose of the experiment described in Phillips et al. The aim is to determine if expression of OPH in the insect cells can enhance detoxication by OPH when insect cells are exposed to an organophosphate insecticide, in order that beneficial insects will not be killed by the insecticide (See p. 8158, column 1 and 2 and especially column 2, first and second full paragraph). Use of OPH as a selectable marker is not disclosed; use of OPH in attempts to keep an insect alive when exposed to organophosphate is the aim.

Furthermore, the authors state that while there are indications OPH may be used in keeping transformed insects alive in the face of exposure to organophosphates, they cannot conclude this can be accomplished, but instead express hope for potential of the idea. Thus they note that, "The modest level of paraoxon resistance resulting from transgenic induction indicates that the existing enzyme could not detoxify paraoxon at expected levels," adding that, "Further work is needed to understand the possible

association of OPH with membranes in *Drosophila* as this association relates to paraoxon resistance." (p. 8158, column 1, second full paragraph).

Therefore, one skilled in the art upon review of Phillips et al. understands that OPH encoding sequences may be transformed into *Drosophila*. Further, OPH was assayed in the fly and found to be expressed. It has potential to prevent toxicity of the insect when exposed to organophosphate insecticides, but further analysis is necessary. The reference does not disclose a method for determining whether a cell has incorporated and expressed a polynucleotide by introducing a construct comprising a polynucleotide encoding an enzyme having OPH activity, contacting the cell with an organophosphate "such that if the cell does not contain the construct and an enzyme having organophosphate hydrolase activity is not thereby produced, the cell growth is inhibited," and thereby determining whether the cell has incorporated the polynucleotide. Inhibition of cell growth used to select for transformed cells is not disclosed in Phillips, and thus claims 1, 13 and dependent claims are not disclosed or anticipated by Phillips. Claim 13 further recites that construct comprises the OPH encoding nucleotide and a first polynucleotide, and determining whether the cell has incorporated first polynucleotide. The heat shock sequences in Phillips et al. are used to determine induction of the OPH gene, not to determine whether the heat shock gene was transformed in to the cells.

Further attenuated are the recitations of claims 7, 19, and 33, which recite a method to determine whether a cell has incorporated and expresses a polynucleotide by introducing a construct comprising a polynucleotide encoding OPH and contacting the cell with organophosphate such that the OPH enzyme hydrolyzes the organophosphate

and detecting the hydrolysis. Thus use as a screenable marker is also possible; a use not suggested or disclosed by Phillips et al.

Section 103 Issues

Claims 1-40 are rejected under section 103 as obvious over Barrett in view of Jilka and Hood et al. The Examiner finds that Barrett teaches transforming plant cells with a polynucleotide encoding P450 enzymes that metabolize organophosphates. The P450 enzymes are used to protect plants from exposure to a pesticide. The Examiner notes Barrett does not teach transforming a plant cell with a nucleotide encoding an organophosphate hydrolase, comprising a first and second polynucleotide, or a polynucleotide encoding SEQ ID NO: 1. The Examiner points to Jilka as showing a plant cell transformed with a polynucleotide encoding organophosphate hydrolase, and to Hood et al as using organophosphate paraoxon to detect OPH activity in a plant. The Examiner finds it obvious to modify the teaching of Barrett to transform a plant with a nucleotide encoding an organophosphate and selecting transformed cells in the presence of an organophosphate that inhibits growth of the cell.

The rejection is respectfully traversed on several grounds. First, Barrett, as the Examiner notes, does not teach a nucleotide sequence encoding organophosphate hydrolase. The patent is instead directed to P450 enzyme encoding sequences, which enzyme can metabolize the herbicides and insecticides. One cannot conclude that the ability of P450 enzymes to metabolize organophosphates demonstrates that an entirely different gene group, those encoding organophosphate hydrolases, will not only metabolize organophosphates, but that the property can be used to produce a selectable or scorable marker. The patent indicates it is possible to determine if a test compound can

protect a plant from deleterious effects of a pesticide, and one method is to detect metabolites following contact with the test compound. As the patent notes, detection of metabolites is routine in the art. The fact one can test for potential protection does not suggest or show with any predictability that a specific protein produced by a gene not only will protect a cell from harmful affects when exposed to a proposed compound, but can also be used as a selectable or screenable marker.

Nor is the invention as claimed suggested by demonstration that nucleotide sequences encoding OPH can be expressed in plants. Many genes can be expressed in plants, but do not provide the properties necessary of being a selectable or screenable marker.

Even accepting the characterization of the references, they, at most, show that (1) P450 enzyme encoding sequences metabolize organophosphates and the metabolites so-produced can be detected; (2) organophosphate hydrolase encoding sequences can be expressed in plants and the activity of OPH detected. If this were the only criteria for identifying a selectable or scorable marker, there would be no limit of such markers available. In fact, there is not. To qualify as a selectable marker, a nucleotide sequence must meet additional criteria. The expression of the gene must be at a level effective in rendering the reagent used for selection harmless and to impact the reagent before it harms the cell. What is more, both the protein and toxin must be able to be in the same part of the cell to interact effectively. The reagent must produce product which will inhibit growth of non-transformed cells. The impact on cell growth of non-transformed cells needs to be such that differentiation between transformed and non-transformed cells is possible. Whether a nucleotide sequence encoding a particular detectable protein is

effective as a selectable marker is not predictable. For example, some markers can themselves have an adverse impact on the expression of a second gene product, as noted with the neomycinphosphotransferase 11 drug resistance gene, found to not be a suitable selectable marker as a result, as discussed at WO 02/57472 (citing Apperly et al, 1991, *Blood* 78:310-317). Some genes used as markers require further steps be employed, as with the mutant variants of human dihydrofolate reductase genes, which require the cell media be depleted of thymidine (WO 02/57472). Thus, the use of a nucleotide sequence as a selectable marker is unpredictable and not obvious.

Additionally, the references do not suggest that OPH genes can be used as a scorable marker, where destruction of the non-transformed cells is not required; instead, hydrolysis of the organophosphate is detected, as recited in claims 7, 19 and 33, and dependent claims. Claims 7 and 19 (and claims depending therefrom) are amended to recite that the hydrolysis of organophosphate is detected "such that extraction of the enzyme from the cell is not necessary to detect the hydrolysis " in order to determine whether the cell has incorporated the polynucleotide of interest "under conditions that do not require destruction of the cell" As recited in amended claims 38-40, such detection can be by spectrophotometry, fluorescence, phosphorescence, and visual observation of the products of the hydrolysis to detect the hydrolysis. The protein of a gene successfully expressed in a plant can typically be detected; but its use as a scorable marker requires more. Here, extraction of the enzyme from the cell is not required for the assay. To qualify as a scorable/screenable marker, the nucleic acid must not only express the protein in plants, but the substrate added must be able to penetrate the cell, and be hydrolyzed such that it can be readily detected, and, here, can be visually observed or

measured without the necessity of destroying the cell or extracting the resultant hydrolysis products or enzyme from the cell. Further, to qualify as a scorable marker, the product produced by hydrolysis must not be lethal to the cell. A marker that is both selectable and scorable is both valuable and unexpected.

Thus, Applicant submits the references do not suggest use of the OPH gene as a selectable or scorable marker, and do not teach a method of determining whether a cell has incorporated and expressed a polynucleotide by introducing the OPH in to the cell, contacting the cell with organophosphate, and either determining if the cell has incorporated the polynucleotide by observing cell growth inhibition or detecting hydrolysis of the organophosphate. For the foregoing reasons, reconsideration and allowance of the claims is respectfully requested. In the event any issues regarding allowance of the claims remain following entry of the amendment, an interview with the Examiner is requested.

Respectfully submitted,

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